Association of urinary phytoestrogen concentrations with serum concentrations of prostate-specific antigen in the National Health and Nutrition Examination Survey

Walser-Domjan, Esther ; Richard, Aline ; Eichholzer, Monika ; Platz, Elizabeth A ; Linseisen, Jakob ; Rohrmann, Sabine

Abstract: Some clinical trials have shown that high phytoestrogen intake may decrease serum concentrations of prostate-specific antigen (PSA), and phytoestrogens may also lower prostate cancer risk. It was the aim of this study to examine the relationship between the serum PSA level and urine phytoestrogen concentration in generally healthy U.S. men. Eight hundred twenty-four men, 40+ yr old without prostate cancer, who participated in the 2001-2004 NHANES surveys, were included in the analysis. The association of total PSA, free PSA, and PSA ratio [free PSA/total PSA * 100] with concentrations of isoflavones and lignans (standardized for urinary creatinine concentration) was examined using multivariable-adjusted linear and logistic regression models. The linear regression analyses showed no clear association between creatinine-standardized urinary phytoestrogen concentrations and serum total or free PSA levels or PSA ratio. However, the odds of having a PSA ratio <15% rose from Quartile 1 to Quartile 4 of isoflavone excretion (odds ratio = 2.82, 95% confidence interval 1.28-6.22 for top vs. bottom quartile), but there were no associations with having a PSA ratio <25%. In generally healthy U.S. men, 40+ yr old without a diagnosis of prostate cancer, urinary isoflavone, and lignan concentrations were not associated with serum PSA level.

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Title

Association of urinary phytoestrogen concentrations with serum concentrations of prostate-specific antigen (PSA) in the National Health and Nutrition Examination Survey (NHANES)

Authors

Esther Walser-Domjan[1], Aline Richard[1], Monika Eichholzer[1], Elizabeth A Platz[2,3,4], Jakob Linseisen[5], Sabine Rohrmann[1]

Affiliations

[1]Division of Cancer Epidemiology and Prevention, Institute of Social and Preventive Medicine, University of Zurich, Zurich, Switzerland

[2]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

[3]Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD

[4]James Buchanan Brady Urological Institute, Johns Hopkins Medical Institutions, Baltimore, MD

[5]Institute of Epidemiology I, Helmholtz Zentrum München, Neuherberg, Germany

Corresponding Author

Sabine Rohrmann

Division of Cancer Epidemiology and Prevention,

Institute of Social and Preventive Medicine,

University of Zurich,

Hirschengraben 84

8002 Zurich,

Switzerland

e-mail: sabine.rohrmann@ifspm.uzh.ch

phone: +41 44 634 5256

Association Phytoestrogens and PSA in NHANES

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Some clinical trials have shown that high phytoestrogen intake may decrease serum concentrations of prostate-specific antigen (PSA), and phytoestrogens may also lower prostate cancer risk. It was the aim of this study to examine the relationship between the serum PSA level and urine phytoestrogen concentration in generally healthy US men. 824 men, 40+ year old without prostate cancer, who participated in the 2001-2004 NHANES surveys, were included in the analysis. The association of total PSA, free PSA, and PSA ratio [free PSA/total PSA * 100] with concentrations of isoflavones and lignans (standardized for urinary creatinine concentration) was examined using multivariable-adjusted linear and logistic regression models. The linear regression analyses showed no clear association between creatinine-standardized urinary phytoestrogen concentrations and serum total or free PSA levels or PSA ratio. However, the odds of having a PSA ratio < 15% rose from quartile 1 to quartile 4 of isoflavone excretion (odds ratio = 2.82, 95 % confidence interval 1.28-6.22 for top versus bottom quartile), but there were no associations with having a PSA ratio < 25%. In generally healthy US men, 40+ years old without a diagnosis of prostate cancer, urinary isoflavone and lignan concentrations were not associated with serum PSA level.

Compared to the United States and Europe, the incidence of prostate cancer is lower in China and Japan. The lower incidence in Asian countries is hypothesized to be at least in part be due to the higher consumption of soy products rich in phytoestrogens in Asian countries (1). Isoflavones and lignans are the most common classes of phytoestrogens in the human diet. The most important sources of isoflavones are soybeans and other soy products, but the major sources of phytoestrogens in the US diet are doughnuts, pancakes, waffles and bread, all of which contain added soy (2). The major isoflavones are genistein, daidzein, formononentin, glycitein and biochanin-A. In 30-50% of the human population the gut microflora can metabolize daidzein to equol; o-desmethylangolensin is another intestinal metabolite formed from daidzein (90% of the human population) (3). Lignan concentrations are very high in flaxseed and pumpkin seed. Smaller amounts are found in a variety of foods including nuts and seeds, legumes, whole grain cereals, vegetables and fruits (2). Lariciresinol and pinoresinol contribute most to total lignan intake, followed by secoisolariciresinol and matairesinol (4). In the human body, these plant lignans are not bioavailable but they can be absorbed in the intestine once they are transformed into enterodiol and enterolactone (2). Phytoestrogens are excreted in the urine (2) and an estimate of alimentary intake of isoflavones can be derived from urinary concentration of isoflavones. Several studies have shown that soy consumption correlates with urinary isoflavone excretion (5), even in spot urine (6). In a Finnish study, nutritional intake of lignans was associated with the urinary excretion of enterolignans (7). Prostate-specific antigen (PSA) is a biochemical marker secreted by the prostate that is used for the diagnosis and surveillance of prostate cancer. Generally, healthy men have a low serum level and higher serum levels of PSA are associated with both localized and advanced prostate cancer. However, PSA is organ-specific but not cancer-specific (8), such that PSA levels can be elevated either because of an enlargement or an inflammation of the prostate (9). Also, serum PSA levels are affected by age (10), body mass index (BMI) (11), ethnicity, (12) circulating levels of C-reactive protein (CRP) (13) and possibly by common drugs including statin drugs (14), thiazide diuretics

(15), nonsteroidal anti-inflammatory drugs (NSAIDs) and acetaminophen (16). With respect to the effect of phytoestrogens, a small prospective Austrian intervention study observed a significant decrease in total PSA levels with daily administration of an isoflavone extract for one year in healthy men with elevated PSA levels (17). In another small double-blind placebo-controlled trial, the level of total PSA was lowered and the PSA ratio was increased in men diagnosed with prostate cancer who consumed bread with different phytoestrogen concentrations (18). Another randomized trial that administered isoflavone supplements showed no change in PSA concentrations (1).

As reviewed above, to date there are only few studies in humans about the possible association of urine concentration or dietary intake of phytoestrogens with serum level of PSA, which yielded partly contradictory results (1, 17-19). It is currently also unclear whether the intake of phytoestrogens with a regular diet in the general population has an effect on PSA concentrations of generally healthy men. Thus, our study offered the opportunity to examine whether phytoestrogen concentration as observed in men with a normal diet were associated with PSA concentration in a large representative sample of US men. We hypothesized that men with higher concentrations of phytoestrogens in the urine have lower serum total PSA level and higher ratio of total to free PSA.

Materials and methods

The National Health and Nutrition Examination Survey (NHANES) is conducted by the US National Center for Health Statistics of the Centers for Disease Control and Prevention. Since 1999 each year data are collected from a representative sample that covers all age groups of the civilian non-institutionalized US population over 2 month of age. The survey is unique because it includes interviews (questionnaires), physical and laboratory examinations.

In the NHANES surveys of the years 2001-2002 and 2003-2004, PSA serum and phytoestrogen urine levels were measured (20). To address our study questions, our study sample included men meeting the following criteria: age 40 years or older, measured blood concentration of PSA,

measured urine concentration of phytoestrogens, never diagnosed with prostate cancer, no recent prostate biopsy, prostate examination or infection prior to venipuncture. The 2001-2002 and 2003-2004 NHANES surveys included 10301 males, of which 3326 were age 40 years or older. PSA has been measured in 2701 men. 155 of them did not meet the prostate exclusion criteria. So, after subtracting those who had no phytoestrogen measurement (N=2546 as phytoestrogens were measured only in a random subsample of the NHANES populations) 824 men were eligible for our study.

PSA, phytoestrogen and creatinine measurement

The Access Hybritech Assay was used to measure concentrations of total and free PSA (Beckman Access, Fullerton CA). The PSA ratio was calculated by dividing the concentration of free PSA by the concentration of total PSA and multiplying by 100 (20, 21).

Spot urine samples were collected in a standardized manner as described previously (22). The analysis of phytoestrogens (daidzein, o-desmethylangolensin equol, genistein, enterodiol, and enterolactone) was carried out with high performance liquid chromatography (HPLC) and tandem mass spectrometric (MS/MS) detection (20, 21). Creatinine concentration, which was used to standardize urinary phytoestrogen, was analyzed using a Jaffe rate reaction measured with a CX3 analyzer (Beckman Instruments, Brea, CA) (22). For the statistical analysis, we evaluated the associations for the sum of isoflavones (daidzein, o-desmethylangolensin equol, genistein), the sum of enterolignans (enterodiol and enterolactone) and the sum of all isoflavones and enterolignans.

Data collection

Age, race/ethnicity, socioeconomic status, smoking status, intake of NSAID and physical activity were determined in a household interview prior to the examination. During a face-to-face interview in the mobile examination center (MEC), questions about alcohol consumption were asked. The

intake of prescribed medication such as statin drugs and thiazide diuretics was assessed by a personal interview by trained NHANES staff during a 1-month period prior to the survey date (23). Trained personnel measured body weight and height in the MEC. Standing height was measured in meters with a stadiometer. The participants were weighted in kilograms using a digital weight scale. Body mass index (BMI) was calculated by dividing the weight in kilograms by the square of the height in meters (24). CRP was measured by the latex-enhanced nephelometry method (21).

Statistical analysis

All statistical analyses accounted for the complex sample design by using the appropriate weights and sampling factors (25). For the characterization of the subpopulation, we computed arithmetic means of all continuous parameters and percentages of all categorical parameters. These calculations were also made by quartiles of genistein urine concentration. Creatinine is a very good parameter for the excretion performance of the kidneys and is used to determine the so called creatinine clearance as measure for the glomerular filtration (26), and both creatinine and phytoestrogen concentrations rise with the excretion performance of the kidneys. Therefore, all analyses were performed by standardizing the phytoestrogen concentration in µg/g creatinine in urine. Linear regression was used to examine the associations of total PSA and PSA ratio serum levels with phytoestrogen urine concentrations. Because both phytoestrogen concentrations in urine and PSA variables were not normally distributed in the study population, these values were transformed using the natural logarithm (14). In the linear regression we controlled for age (continuous), race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, other), C-reactive protein (continuous), pain relievers (taken nearly every day for a month or longer yes/no), statin drugs (used in the past month yes/no), thiazide diuretics (used in the past month yes/no), smoking (ever smokers, never smokers), alcohol consumption (missing, abstinent, <1 drink per day, 1-2 drinks per day, e 3 drinks per day), poverty-income ratio (0-0.99 = below poverty, e 1 =

150 at or above poverty) (27), physical activity (any vigorous activities over past 30 days for at least 10
151 minutes that caused heavy sweating or large increases in breathing or heart rate, 1 = yes, 0 = no)
152 (28), BMI (continuous), educational level (<high school, High School Diploma (including GED
153 [General Education Development]), >high school, refused/don't know/missing). In linear models,
154 where both the dependent and the independent variables have been ln-transformed, the dependent
155 variable can be interpreted as percent changes for a one-percent increase in the independent variable
156 while all other variables in the model are held constant. In addition to linear models, logistic
157 regression models were used to test if there is an association between urine concentrations of
158 phytoestrogens and abnormal levels of total PSA and PSA ratio. For the logistic regression, the
159 outcome variable *total PSA* was converted into a categorical variable using 2.5 and 4.0 ng/ml as
160 threshold values, which have been used for prostate cancer detection in different publications (29-
161 31). A lower PSA ratio is suggestive of prostate cancer. In our study, *PSA ratio* was converted into
162 a categorical variable using, as proposed in some studies, 15% as threshold value (32, 33) and 25%
163 as an alternative threshold, which was proposed in other publications (32, 33). Phytoestrogen urine
164 concentrations were categorized into quartiles. We controlled for the above-mentioned confounders
165 in the logistic regression models.
166 Stata IC/ 11.2 software (College Station, Texas) was used to perform the analyses.
167 The Institutional Review Board of the National Center for Health Statistics, Centers for Disease
168 Control and Prevention, approved all protocols for the implementation of NHANES 2001-2004.
169 Informed consent was obtained for all participants (14).
170
171 Results
172 Table 1 shows the baseline characteristics of the study population. Mean age of the participants was
173 54.6 years; mean BMI was in the overweight range (28.8 kg/m^2). Most men were non-Hispanic
174 White (58%), were married (68.6%), and 47.6% of men had an education level higher than high

175 school or GED. Fourteen percent of the study population lived below poverty level. The majority of
176 men were ever smokers (71.6%) and 43.5% of the men drank more than one alcoholic drink per day
177 in the past 12 months prior to the survey date.
178 Mean total and free PSA serum concentration were in the normal range (1.5 (standard error [SE] ±
179 0.1) and 0.4 (± 0.1) ng/ml, respectively); mean PSA ratio was 30.2% (± 0.6%). Mean genistein,
180 enterodiol and enterolactone concentrations were 190.4 (± 27.1), 146.9 (± 41.8) and 910.7 (± 104.7)
181 ng/ml, respectively.
182 In the linear regression models, the associations of phytoestrogen concentrations with total or free
183 PSA serum levels and PSA ratio were not statistically significant (Table 2).
184 There was no clear pattern of the association between urinary phytoestrogen concentrations and
185 odds of having elevated total PSA concentration, and none of the associations were statistically
186 significant (Table 3). However, the sum of isoflavones and the sum of all phytoestrogens were
187 associated with an increased odds of having a PSA ratio < 15%, when comparing top versus bottom
188 quartiles. For example, men in the top quartile of the sum of isoflavones had an OR = 2.82 (95% CI
189 1.28-6.22) of having a PSA ratio < 15% compared with men in the bottom quartile. In contrast,
190 there were no statistically significant associations between urine isoflavone levels and odds of
191 having PSA ratio < 25%.
192 In sub-analyses, we computed linear regressions for the subgroup of the men without limited renal
193 function (which was defined as not having a chronic kidney disease: glomerular filtration rate
194 e 60ml/min/1.73m^2; unweighted n=320; data not shown) (34). The results of the linear regressions
195 of the subgroup were similar to the results of the linear regression of the main sample and not
196 statistically significant.
197
198 Discussion

A PSA-decreasing effect of phytoestrogens has been observed in cell cultures and mice (35-39). In LNCaP cells, genistein abrogated the stimulation of PSA by 17² -estradiol, and equol administration reduced PSA levels (35, 39). Some human feeding studies, which examined participants with pathological changes in the tissue of the prostate, have shown declines in PSA levels with phytoestrogen intake (17, 18, 40) or administration of soy milk (19), whereas another human feeding study found no significant effect of soy consumption on serum total or free PSA levels in healthy men (41). Also, in a double-blind, placebo-controlled randomized trial among men with prostate cancer, the administration of isoflavone supplements did not change PSA concentrations (42), but another soy intervention study observed a decrease of PSA concentrations (43). However, to the best of our knowledge, our study is the first effort to investigate the cross-sectional association between urinary phytoestrogen concentration and serum PSA level in a group of men of the general US population. We observed no clear association between urinary phytoestrogen concentration and having elevated total or free PSA concentration in the examined population. However, men in the top quartiles of isoflavones and the sum of all phytoestrogens, i.e., isoflavones and lignans, had an elevated odds of having a PSA ratio below 15%. However, there was no statistically significant odds ratio when using a cut point of 25%.

The anticancer potential of the isoflavone tectorigenin was demonstrated on the molecular level (44) and the chemopreventive potential of genistein against prostate cancer was shown in two animal models (45). The anti prostate cancer effect of the phytoestrogen group of isoflavones could be hypothesized in the possible action of isoflavones on the androgen receptor (AR) in prostate tumor cells (19). Additionally the isoflavone genistein is able to inhibit several cancer promoting factors on the molecular level (1). A meta-analysis of 8 studies (US, Canada, Japan, China and Taiwan) reported an inverse association of soy food consumption with the risk for prostate cancer. (46) Moreover, a nested case-control study among Japanese men observed that serum daidzein, genistein and equol seemed to dose-dependently reduce prostate cancer risk (47). However, in our

224 study, the outcome was circulating level of PSA, which in the US has been used as a screening
225 instrument for prostate cancer. PSA, however, is not cancer-specific; higher levels of serum PSA
226 are also observed in benign prostate conditions such as benign prostatic hyperplasia and prostatitis.
227 In evaluating whether phytoestrogens influence the likelihood of an abnormal PSA result, we used a
228 PSA cut point of 4 ng/ml, which is often used as threshold value for prostate biopsy (48), and of 2.5
229 ng/ml, which is sometimes used clinically for prostate cancer screening (29, 49), but we observed
230 no statistically significant associations for either cut-point. In contrast, we observed an increased
231 odds of having a PSA ratio < 15% with increasing isoflavone excretion. However, there were no
232 associations when using a PSA ratio of 25% as a cut-off and we cannot exclude that the first
233 observation is merely a chance finding.
234 PSA screening is not optimal for detecting prostate cancer, with the consequence of possible
235 overtreatment of men who have a pathological PSA but who have no prostate cancer or, on the
236 other hand a prostate cancer that remains clinical unapparent (50, 51). Several factors also influence
237 the PSA level. Besides inflammation of the prostate, urinary retention, ejaculation and ambulation
238 have an influence on the PSA value (52).
239 Compared to the data of the study of Valentín-Blasini et al. (53), phytoestrogen concentrations in
240 this subpopulation of US men 40+ years old were in almost the same range. However, urinary
241 levels of isoflavones of our sample were lower than measured in Vietnamese and Japanese samples
242 (54). Thus, our results may indicate that the range of isoflavone intake in a general group of US
243 men might be too narrow and too low to significantly affect PSA concentration in healthy men. An
244 analysis of the Asian subgroup in our sample was not possible due to a small sample size (1.7% of
245 the study population). On the other hand, levels of lignans of our sample were higher compared to
246 the samples of the Asian study (54), supporting the importance of looking at lignan levels in
247 addition to isoflavones in European and North-American societies.

The cross-sectional study design is one of the limitations of our study. To test whether phytoestrogen consumption affects PSA concentration, multiple measurements would better reflect actual PSA and phytoestrogen levels. Indeed, a decrease in the rate of rise of serum PSA levels in men with PSA recurrent disease was shown in two trials (19, 55), but a third trial did not report any association (1). However, the number of participants in these trials was limited and larger prospective studies are warranted.

A further limitation is the use of spot urine samples for the determination of phytoestrogen levels. Measurements in spot urines may not be representative for the habitual nutritional intake of phytoestrogens. The half-lives of phytoestrogens are in the range of 3-10 hours (53). However, in a previous analysis of NHANES III data, urinary and serum levels of phytoestrogens showed a good correlation (daidzein r=0.72) (53). In addition, the problematic accuracy of PSA such that changes in PSA concentration over time can either be physiological or pathological has been discussed in different publications (1, 19, 56).

In conclusion, the results of this study based on the 2001-2004 NHANES surveys, a population with a generally low intake of phytoestrogens compared with, e.g. Asian populations, showed no clear relationship between serum PSA level and urinary phytoestrogen concentration. We observed an increased odds of having a PSA ratio < 15% with high phytoestrogen levels, but there was no association when using a different cut-point for categorizing PSA ratio. Longitudinal studies among healthy men, preferably with multiple measurements of phytoestrogens and PSA, may be better suited to address the question whether habitual phytoestrogen intake has an effect on PSA concentration than cross-sectional studies.

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278 Table 1. Baseline characteristics by quartiles of urine genistein concentration, men 40+ years old in NHANES 2001-2004

Characteristic	Mean / percentage *†	SE of mean
	N=824	
Age (years)	54.6	0.4
BMI (kg/m^2)	28.8	0.3
Race (%)		
Non-Hispanic white	58.0	
Non-Hispanic black	18.5	
Hispanic	21.8	
Other	1.7	
Education level (%)**		
<High school	29.4	
High school/GED	22.9	
>High School	47.6	
Living below poverty (PIR < 1) (%)	14.0	
Smoking history – ever smoker (%)	71.6	
Average number of alcoholic drinks/day –past 12 months (%)**		
0	32.2	
d1	21.5	
>1	43.5	
Any vigorous activity over past 30 days (%)	27.2	
Use of pain relievers (NSAIDs) nearly every day for a month or longer (%)	30.6	
Statin use, past month (%)	12.0	
Thiazide diuretic use, past month (%)	9.0	
Creatinine, urine (mg/dl)	143.6	3.1
C-reactive protein (mg/dl)	0.4	0.1
Total PSA (ng/ml)	1.5	0.1
Free PSA (ng/ml)	0.4	0.1
PSA ratio (%)	30.2	0.6
Isoflavones		
Daidzein (ng/ml)	318.0	46.1
Genistein (ng/ml)	190.4	27.1
o-Desmethylangolensin (ng/ml)	45.1	7.7
Equol (ng/ml)	21.1	5.3
Lignans		
Enterodiol (ng/ml)	146.9	41.8
Enterolactone (ng/ml)	910.7	104.7

279 BMI, body mass index; GED, General Education Development; PIR, Poverty Income Ratio; SE, standard error.

280 * Values are means or percentages unless otherwise noted.

281 † The sum of the percentages may not always be 100 due to rounding.

282 ** Does not add up to 100% because of missing information.

283

Table 2. Linear regression: outcome variables total PSA, free PSA and PSA ratio levels in the serum, exposure variables creatinine-adjusted phytoestrogen-levels in the urine controlled for confounders†; NHANES 2001-2004

Phytoestrogen‡	Beta-coefficient	SE	Change in % per 1% change in total or free PSA concentration of PSA ratio	
			change in %	95% CI
Total PSA concentration§				
Sum of isoflavones	-0.008	0.026	-0.80	-5.73 to 4.39
Sum of lignans	0.013	0.02	1.31	-2.59 to 5.36
Sum of phytoestrogens	-0.012	0.022	-1.19	-5.36 to 3.16
Free PSA concentration				
Sum of isoflavones	-0.007	0.013	-0.7	-3.2 to 1.9
Sum of lignans	0.0001	0.009	0.01	-1.7 to 1.8
Sum of phytoestrogens	-0.003	0.007	-0.3	-1.7 to 1.1
PSA ratio (%)§				
Sum of isoflavones	0.011	0.014	1.11	-1.63 to 3.92
Sum of lignans	0.001	0.014	0.10	-2.61 to 2.88
Sum of phytoestrogens	0.011	0.015	1.11	-1.82 to 4.12

CI, confidence interval; SE, standard error

*P< 0.05

† Adjusted for age, race/ethnicity, poverty-income-ratio, educational level, BMI, C-reactive protein, NSAIDs, statins, thiazide diuretics, smoking, alcohol-consumption, physical activity

‡ Units of creatinine-adjusted phytoestrogen concentration were per 500µg/g creatinine, urine

§ PSA-level units: The natural logarithm of total PSA in ng/ml, of free PSA in ng/ml and of PSA Ratio in %

293 Table 3. Association between quartiles of urine phytoestrogen concentration* and elevated total PSA concentration or PSA ratio,
294 NHANES 2001-2004†

	Quartiles of phytoestrogen concentration							
	1	2		3		4		
	OR	OR	95% CI	OR	95% CI	OR	95% CI	p-trend
Total PSA >2.5ng/ml								
Sum of isoflavones	1.00	0.80	0.40,1.60	0.88	0.47,1.67	0.84	0.37,1.87	0.65
Sum of lignans	1.00	1.70	0.78,3.70	2.18	0.93,5.12	1.69	0.65,4.41	0.26
Sum of phytoestrogens	1.00	1.06	0.46,2.45	0.96	0.37,2.49	1.11	0.47,2.64	0.88
Total PSA >4.0ng/ml								
Sum of isoflavones	1.00	1.05	0.60,1.84	1.59	0.85,2.99	1.31	0.44,3.88	0.39
Sum of lignans	1.00	1.81	0.41,8.05	3.25	0.83,12.7	2.17	0.46,10.3	0.25
Sum of phytoestrogens	1.00	1.19	0.45,3.12	1.33	0.45,3.97	1.33	0.37,4.85	0.66
PSA ratio <15%								
Sum of isoflavones	1.00	1.04	0.39,2.82	1.52	0.67,3.44	2.82	1.28,6.22	0.02
Sum of lignans	1.00	0.45	0.12,1.76	1.02	0.35,2.95	2.30	0.88,5.99	0.06
Sum of phytoestrogens	1.00	2.40	0.79,7.29	1.65	0.55,4.90	3.77	1.34,10.6	0.03
PSA ratio <25%								
Sum of isoflavones	1.00	0.85	0.51,1.41	0.91	0.49,1.69	0.99	0.50,1.97	0.99
Sum of lignans	1.00	0.68	0.43,1.08	0.75	0.42,1.33	1.00	0.60,1.67	0.95
Sum of phytoestrogens	1.00	0.92	0.54,1.58	0.91	0.54,1.54	1.08	0.64,1.81	0.81

295 CI, confidence interval.

296 * Phytoestrogen concentration units: µg/g creatinine, urine

297 † Adjusted for age, race/ethnicity, C-reactive protein, NSAIDs, statins, thiazide diuretics, smoking, alcohol-consumption, poverty-
298 income-ratio, physical activity, BMI, educational level

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300

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References

1. deVere White, RW, Tsodikov, A, Stapp, EC, et al. Effects of a high dose, aglycone-rich soy extract on prostate-specific antigen and serum isoflavone concentrations in men with localized prostate cancer. Nutr Cancer 2010;62*:* 1036-43.
2. Valentin-Blasini, L, Sadowski, MA, Walden, D, et al. Urinary phytoestrogen concentrations in the U.S. population (1999-2000). J Expo Anal Environ Epidemiol 2005;15*:* 509-23.
3. Atkinson, C, Frankenfeld, CL, and Lampe, JW. Gut bacterial metabolism of the soy isoflavone daidzein: exploring the relevance to human health. Exp Biol Med (Maywood) 2005;230*:* 155-70.
4. Milder, IE, Feskens, EJ, Arts, IC, et al. Intake of the plant lignans secoisolariciresinol, matairesinol, lariciresinol, and pinoresinol in Dutch men and women. J Nutr 2005;135*:* 1202-7.
5. Maskarinec, G, Singh, S, Meng, L, et al. Dietary soy intake and urinary isoflavone excretion among women from a multiethnic population. Cancer Epidemiol Biomarkers Prev 1998;7*:* 613-9.
6. Seow, A, Shi, CY, Franke, AA, et al. Isoflavonoid levels in spot urine are associated with frequency of dietary soy intake in a population-based sample of middle-aged and older Chinese in Singapore. Cancer Epidemiol Biomarkers Prev 1998;7*:* 135-40.
7. Nurmi, T, Mursu, J, Penalvo, JL, et al. Dietary intake and urinary excretion of lignans in Finnish men. Br J Nutr 2010;103*:* 677-85.
8. Cotran, R, V, K, and T, C. The Male Genital Tract. Robbins Pathologic Basis of Disease Sixth Edition, pp. 1011-1034. Philadelphia: W.B. Saunders Company, 1999.
9. Arcangeli, CG, Ornstein, DK, Keetch, DW, et al. Prostate-specific antigen as a screening test for prostate cancer. The United States experience. Urol Clin North Am 1997;24*:* 299-306.
10. Kristal, AR, Chi, C, Tangen, CM, et al. Associations of demographic and lifestyle characteristics with prostate-specific antigen (PSA) concentration and rate of PSA increase. Cancer 2006;106*:* 320-8.
11. Culp, S, and Porter, M. The effect of obesity and lower serum prostate-specific antigen levels on prostate-cancer screening results in American men. BJU Int 2009;104*:* 1457-61.
12. He, D, Wang, M, Chen, X, et al. Ethnic differences in distribution of serum prostate-specific antigen: a study in a healthy Chinese male population. Urology 2004;63*:* 722-6.
13. Lehrer, S, Diamond, EJ, Mamkine, B, et al. C-reactive protein is significantly associated with prostate-specific antigen and metastatic disease in prostate cancer. BJU Int 2005;95*:* 961-2.
14. Mondul, AM, Selvin, E, De Marzo, AM, et al. Statin drugs, serum cholesterol, and prostate-specific antigen in the National Health and Nutrition Examination Survey 2001-2004. Cancer Causes Control 2010;21*:* 671-8.
15. Chang, SL, Harshman, LC, and Presti, JC, Jr. Impact of common medications on serum total prostate-specific antigen levels: analysis of the National Health and Nutrition Examination Survey. J Clin Oncol 2010;28*:* 3951-7.
16. Singer, EA, Palapattu, GS, and van Wijngaarden, E. Prostate-specific antigen levels in relation to consumption of nonsteroidal anti-inflammatory drugs and acetaminophen: results from the 2001-2002 National Health and Nutrition Examination Survey. Cancer 2008;113*:* 2053-7.
17. Engelhardt, PF, and Riedl, CR. Effects of one-year treatment with isoflavone extract from red clover on prostate, liver function, sexual function, and quality of life in men with elevated PSA levels and negative prostate biopsy findings. Urology 2008;71*:* 185-90; discussion 190.
18. Dalais, FS, Meliala, A, Wattanapenpaiboon, N, et al. Effects of a diet rich in phytoestrogens on prostate-specific antigen and sex hormones in men diagnosed with prostate cancer. Urology 2004;64*:* 510-5.
19. Pendleton, JM, Tan, WW, Anai, S, et al. Phase II trial of isoflavone in prostate-specific antigen recurrent prostate cancer after previous local therapy. BMC Cancer 2008;8*:* 132.
20. National Health and Nutrition Examination Survey Data. 2011. Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. National Center for Health Statistics (NCHS), Centers for Disease Control and Prevention. National Center for Health Statistics (NCHS) 2011.

21. National Health and Nutrition Examination Survey Laboratory Protocol. 2011. Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. National Center for Health Statistics (NCHS), Centers for Disease Control and Prevention. National Center for Health Statistics (NCHS) 2011.
22. Frankenfeld, CL. Dairy consumption is a significant correlate of urinary equol concentration in a representative sample of US adults. Am J Clin Nutr 2011;93*:* 1109-16.
23. National Health and Nutrition Examination Survey Questionnaire 2011. Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. National Center for Health Statistics (NCHS), Centers for Disease Control and Prevention. National Center for Health Statistics (NCHS) 2011.
24. National Health and Nutrition Examination Survey Examination Protocol. 2011. Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. National Center for Health Statistics (NCHS), Centers for Disease Control and Prevention. National Center for Health Statistics (NCHS) 2011.
25. Welch, AA, Lund, E, Amiano, P, et al. Variability of fish consumption within the 10 European countries participating in the European Investigation into Cancer and Nutrition (EPIC) study. Public Health Nutr 2002;5*:* 1273-85.
26. Herold, G. INNERE MEDIZIN 2002. Cologne: Gerd Herold, 2002.
27. Statistics, NCfH. Analytic and Reporting Guidelines: The Third National Health and Nutrition Examination Survey, NHANES III (1988-94). 2012: National Center for Health Statistics, Centers for Disease Control and Prevention, 1996.
28. National Center for Health Statisitcs, CfDCaP. 2001-2002 Data Documentation, Codebook, and Frequencies. 2012: National Center for Health Statisitcs, Centers for Disease Control and Prevention, 2004.
29. Stephan, C, Miller, K, and Jung, K. Is there an optimal prostate-specific antigen threshold for prostate biopsy? Expert Rev Anticancer Ther 2011;11*:* 1215-21.
30. Welch, HG, Schwartz, LM, and Woloshin, S. Prostate-specific antigen levels in the United States: implications of various definitions for abnormal. J Natl Cancer Inst 2005;97*:* 1132-7.
31. Greene, KL, Albertsen, PC, Babaian, RJ, et al. Prostate specific antigen best practice statement: 2009 update. J Urol 2009;182*:* 2232-41.
32. Lacher, DA, Thompson, TD, Hughes, JP, et al. Total, free, and percent free prostate-specific antigen levels among U.S. men, 2001-04. Adv Data 2006*:* 1-12.
33. Catalona, WJ, Partin, AW, Slawin, KM, et al. Use of the percentage of free prostate-specific antigen to enhance differentiation of prostate cancer from benign prostatic disease: a prospective multicenter clinical trial. JAMA 1998;279*:* 1542-7.
34. Levey, AS, Eckardt, KU, Tsukamoto, Y, et al. Definition and classification of chronic kidney disease: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). Kidney Int 2005;67*:* 2089-100.
35. Smith, S, Sepkovic, D, Bradlow, HL, et al. 3,3'-Diindolylmethane and genistein decrease the adverse effects of estrogen in LNCaP and PC-3 prostate cancer cells. J Nutr 2008;138*:* 2379-85.
36. Han, HY, Wang, XH, Wang, NL, et al. Lignans isolated from Campylotropis hirtella (Franch.) Schindl. decreased prostate specific antigen and androgen receptor expression in LNCaP cells. J Agric Food Chem 2008;56*:* 6928-35.
37. Lazarevic, B, Karlsen, SJ, and Saatcioglu, F. Genistein differentially modulates androgen-responsive gene expression and activates JNK in LNCaP cells. Oncol Rep 2008;19*:* 1231-5.
38. Bylund, A, Zhang, JX, Bergh, A, et al. Rye bran and soy protein delay growth and increase apoptosis of human LNCaP prostate adenocarcinoma in nude mice. Prostate 2000;42*:* 304-14.
39. Lund, TD, Blake, C, Bu, L, et al. Equol an isoflavonoid: potential for improved prostate health, in vitro and in vivo evidence. Reprod Biol Endocrinol 2011;9*:* 4.
40. Lazarevic, B, Boezelijn, G, Diep, LM, et al. Efficacy and safety of short-term genistein intervention in patients with localized prostate cancer prior to radical prostatectomy: a randomized, placebo-controlled, double-blind Phase 2 clinical trial. Nutr Cancer 2011;63*:* 889-98.

41. Jenkins, DJ, Kendall, CW, D'Costa, MA, et al. Soy consumption and phytoestrogens: effect on serum prostate specific antigen when blood lipids and oxidized low-density lipoprotein are reduced in hyperlipidemic men. J Urol 2003;169*:* 507-11.
42. Izumi, T, Piskula, MK, Osawa, S, et al. Soy isoflavone aglycones are absorbed faster and in higher amounts than their glucosides in humans. J Nutr 2000;130*:* 1695-9.
43. Maskarinec, G, Morimoto, Y, Hebshi, S, et al. Serum prostate-specific antigen but not testosterone levels decrease in a randomized soy intervention among men. Eur J Clin Nutr 2006;60*:* 1423-9.
44. Thelen, P, Seseke, F, Ringert, RH, et al. [Pharmacological potential of phytoestrogens in the treatment of prostate cancer]. Urologe A 2006;45*:* 195-6, 197-201.
45. Lamartiniere, CA, Cotroneo, MS, Fritz, WA, et al. Genistein chemoprevention: timing and mechanisms of action in murine mammary and prostate. J Nutr 2002;132*:* 552S-558S.
46. Yan, L, and Spitznagel, EL. Meta-analysis of soy food and risk of prostate cancer in men. Int J Cancer 2005;117*:* 667-9.
47. Ozasa, K, Nakao, M, Watanabe, Y, et al. Serum phytoestrogens and prostate cancer risk in a nested case-control study among Japanese men. Cancer Sci 2004;95*:* 65-71.
48. Porter, MP, Stanford, JL, and Lange, PH. The distribution of serum prostate-specific antigen levels among American men: implications for prostate cancer prevalence and screening. Prostate 2006;66*:* 1044-51.
49. Lin, K, Lipsitz, R, Miller, T, et al. Benefits and harms of prostate-specific antigen screening for prostate cancer: an evidence update for the U.S. Preventive Services Task Force. Ann Intern Med 2008;149*:* 192-9.
50. Chou, R, Croswell, JM, Dana, T, et al. Screening for Prostate Cancer; A Review of the Evidence for the U.S. Preventive Services Task Force. Ann Intern Med 2011;155*:* 762-771.
51. Screening for prostate cancer: U.S. Preventive Services Task Force recommendation statement. Ann Intern Med 2008;149*:* 185-91.
52. Tchetgen, MB, and Oesterling, JE. The effect of prostatitis, urinary retention, ejaculation, and ambulation on the serum prostate-specific antigen concentration. Urol Clin North Am 1997;24*:* 283-291.
53. Valentin-Blasini, L, Blount, BC, Caudill, SP, et al. Urinary and serum concentrations of seven phytoestrogens in a human reference population subset. J Expo Anal Environ Epidemiol 2003;13*:* 276-82.
54. Kunisue, T, Tanabe, S, Isobe, T, et al. Profiles of Phytoestrogens in Human Urine from Several Asian Countries. J Agric Food Chem 2010;58*:* 9838-46.
55. Hussain, M, Banerjee, M, Sarkar, FH, et al. Soy isoflavones in the treatment of prostate cancer. Nutr Cancer 2003;47*:* 111-7.
56. Sighinolfi, MC, Micali, S, De Stefani, S, et al. Retrospective descriptive analysis of the physiological kinetics of prostate-specific antigen in men older than 75 years. Asian J Androl 2009;11*:* 493-7.